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(54) Title: NEW NUCLEAR RECEPTOR COFACTORS AND RELATED MODULATORS

(57) Abstract: The invention relates to nuclear receptor cofactors, to methods for identifying modulators of nuclear receptor activity and to methods for treating and/or preventing or pathologic conditions associated with cell types that express said nuclear receptors. In particular, the invention concerns cofactors of PPAR-gamma (PPARγ), screeing methods that can be used for the identification of compounds useful for modulating the PpAR-gamma biological activity and methods of treatment and/or prevention of various PPAR-gamma related diseases and conditions, including metabolic or cell proliferative disorders.

New nuclear receptor cofactors and related modulators

The invention relates to nuclear receptor cofactors, to methods for identifying modulators of nuclear receptor activity and to methods for treating and/or preventing diseases or pathologic conditions associated with cell types that express said nuclear receptors. In particular, the invention concerns cofactors of PPAR-gamma (PPARγ), screening methods that can be used for the identification of compounds useful for modulating the PPAR-gamma biological activity and methods of treatment and/or prevention of various PPAR-gamma related diseases and conditions, including metabolic or cell proliferative disorders.

The following description is provided to aid in understanding the invention but is not admitted to be prior art to the invention.

Peroxisome proliferator-activated receptors (PPARs) are transcription factors that belongs to the nuclear hormone receptor superfamily. These receptors function as ligand-activated transcription factors that control the expression of target genes by binding as heterodimers with the retinoid X receptors (RXRs) to cognate sequences (PPREs) in the promoter regions of those target genes. The first PPAR target genes identified were found to encode mainly enzymes involved in glucose, lipid, and cholesterol metabolism. However, further investigations have shown that PPARs have pleiotropic biological activities and wide-ranging medical applications, extending from uses in metabolic disorders to possible applications in inflammation and cancer (Spiegelman, 1998, Diabetes, 47, 507-514 ; Schoonjans et al., 1997, Curr. Opin. Lipidol., 8, 159-166). Those skilled in the art will appreciate the numerous additional examples of PPAR mediated diseases and pathologic conditions that have been described in literature (see below). For example, the discovery that these transcription factors are involved in the control of lipid metabolism has provided new insights into the

regulation of vertebrate energy homeostasis, and further has provided new molecular targets for the development of therapeutic agents for disorders such as obesity, diabetes and dyslipidemia and related conditions.

The PPAR subfamily includes three subtypes, i.e. PPAR-alpha, PPAR-gamma and PPAR-beta that have distinct tissue expression patterns and exert different physiological roles. PPAR-alpha (PPARα or NR1C1) is highly expressed in the liver, skeletal muscle, kidney and heart, and stimulates the expression of several enzymes involved in peroxisomal beta-oxidation. In addition to being activated by a variety of medium and long-chain fatty acids, PPAR-alpha was found to be the molecular target of the fibrate class of hypolipidemic drugs, such as clofibrate (i.e. 2-(4-chlorophenoxy)-2-methylpropanoic acid ethyl ester), fenofibrate (i.e. 2-(4-(4-chlorobenzoyl)phenoxy)-2-methylpropanoic acid isopropyl ester), bezafibrate (i.e. 2-(4-(4-chlorobenzoylaminoethyl)phenoxy)-2-methylpropanoic acid), ciprofibrate (i.e. 2-(4-(2,2-dichlorocyclopropyl)phenoxy)isobutyric acid) , beclofibrate and etofibrate, as well as gemfibrozil (i.e. 2-(2,4-dimethylphenoxypropyl)-2-methylpropanoic acid) (Fruchart, 2001, Am. J. Cardiol., 88, 24N-29N). Examples of PPAR-alpha ligands are provided in US 6,071,955.

PPAR-gamma (PPARγ or NR1C3) is most abundantly expressed in adipose tissues, the large intestine, and cells of the monocyte lineage. PPAR-gamma plays a central role in adipogenesis, the regulation of fatty acid storage in adipose tissue, insulin sensitization and in the control of circulating glucose levels. PPAR-gamma has been reported to affect cell proliferation, differentiation (e.g. adipocyte differentiation) and apoptosis pathways. Further evidence is accumulating that suggests an important role for PPAR-gamma in atherosclerosis, inflammation and cancer (for a review, Fajas et al., 2001, J. Mol. Endo., 27, 1-9 or Rosen et al., 2000, Genes & Dev 14, 1293-1307, herein incorporated by reference). PPAR-gamma ligands include prostanoids, fatty acids, thiazolidinediones and N-(2-benzoylphenyl)tyrosine analogues which improve metabolic abnormalities associated with type 2 diabetes, such as hyperglycemia,

hyperlipidemia, insulin resistance and other cardiovascular risk factors (Lenhard, 2001, Receptors Channels , 7, 249-58). The DNA sequences for the PPAR-gamma receptors have been described in Elbrecht, et al., 1996, BBRC 224, 431-437, and are herein incorporated by reference (see also reference P37231 of NCBI data base).

PPAR-beta (PPARβ or NR1C2; also known as PPAR-delta, NUC-1 or FAAR) is ubiquitously expressed and its role in mammalian physiology is still largely undefined. However, Oliver et al. (2001, Proc. Natl. Acad. Sci., 98, 5306-11) have recently demonstrated that PPAR-beta is implicated in the regulation of reverse cholesterol transport and Michalik et al. (2000, Horm. Res., 54, 263-268) have shown that PPAR-beta is implicated in the control of keratinocyte proliferation and is necessary for rapid healing of a skin wound. The human DNA sequences for the PPAR-beta has been cloned and is fully described in Schmidt et al., 1992, Molecular Endocrinology, 6, 1634-1641, and is herein incorporated by reference.

Literature provides numerous examples illustrating that PPARs are closely involved in a wide array of diseases or pathological conditions which are associated with cells expressing these nuclear receptors. More specifically, PPARs are useful as drug target in methods for reducing blood glucose, cholesterol and triglyceride levels and are accordingly explored for the treatment and/or prophylaxis of insulin resistance (type 2 diabetes ; see for example WO 98/05331), impaired glucose tolerance, dyslipidemia, and other disorders related to Syndrome X (WO 97/25042, WO 97/10813, WO 97/28149 ; see also Kaplan et al., 2001, J Cardiovasc Risk, 8, 211-7) including hypertension, obesity, atherosclerosis, thrombosis (Duez et al., 2001, J. Cardiovasc. Risk, 8, 185-186), coronary artery disease and other cardiovascular disorders. Further, PPARs have been shown to be potential targets for the treatment of inflammatory diseases such as cutaneous disorders (including acne vulgaris, cutaneous disorders with barrier dysfunction, cutaneous effects of aging, poor wound healing associated with altered signal transduction ; see Smith et al., 2001, J. Cutan. Med. Surg., 5, 231-43), gastrointestinal diseases (WO 98/43081) or renal diseases including glomerulonephritis,

glomerulosclerosis, nephrotic syndrome, hypertensive nephrosclerosis ; similarly PPARs are useful for improving cognitive functions in neurologic diseases (Landreth and Heneka, 2001, Neurobiol Aging, 22, 937-44) or in dementia, for treating diabetic complications, psoriasis, polycystic ovarian syndrome (PCOS) or for preventing and treating bone loss, e.g. osteoporosis ; or for antiviral, antiproliferative or antitumoral treatments (see for example US 5,981,586 or US 6,291,496).

Thus, it is obvious that the PPARs are exciting targets for the development of therapeutic compounds likely to have utility in the treatment of diseases that involve insulin sensitivity, lipid and glucose homeostasis, as well as vascular or inflammatory diseases or disorders. Although, the responses observed in the context of these various treating and/or preventing methods, are encouraging (for example, the thiazolidinedione (TZD) class of medication, e.g. troglitazone, rosiglitazone or pioglitazone, unambiguously plays a critical role in improving insulin sensitivity in patients with type 2 diabetes ; see Cheng lai and Levine, 2000, Heart Dis., 2, 326-333), they are not fully satisfactory treatments because of the occurrence of undesirable side effects (for example, weigh gain, hypertension, cardiac hypertrophy, haemodilution, liver toxicity, oedema ; see Haskins et al., 2001, Arch Toxicol. , 75, 425-438 ; Yamamoto et al., 2001, Life Sci., 70, 471-482 ; Scheen, 2001, Diabetes Metab., 27, 305-313 ; Gale, 2001, Lancet, 357, 1870-1875 ; Forman et al., 2000, Ann. Intern. Med., 132, 118-121 and Al-Salman et al., 2000, Ann. Intern. Med., 132, 121-124). Consequently, it is desirable to have novel improved products and/or novel methods which enable the treatment and/or the prevention of diseases or pathological conditions associated with cell types that express PPAR nuclear receptors. More specifically, most of the side effects observed with TZD derivatives are attributable to the full-agonist property of said compounds, thus it is desirable to identify new compounds that are not necessarily full-agonist. Similarly, it is desirable to have methods for identifying, characterizing and/or evaluating said types of products and methods.

It is widely acknowledged that nuclear receptors, such as PPAR-gamma, achieve trancriptional activation or repression by binding to cognate sequences in the promoter regions of target genes and by recruiting numerous cofactor complexes whose activities range from chromatin remodeling, histone and cofactor modification, to basic transcription machinery recruitment (Glass, & Rosenfeld, 2000, Genes Dev., 14, 121-141). These cofactors partially determine the specificity of action of nuclear receptors and integrate their action in a network of stimuli whose proper orchestration leads to a specific cellular response. Hence, the determination of the multiple partnerships in which each nuclear receptor is engaged, as a function of time and cell type, is a crucial aspect leading to a better understanding of the activity of nuclear receptors on transcriptional regulation. For instance, it is known that for certain hormones, such as estrogen, the response to the hormone is determined almost to the same extent by the presence of the respective nuclear hormone receptor, as by the presence of the cofactors, which interact with the receptors. Various PPAR cofactors have been identified so far. Some cofactors such as p300/CBP (Dowell et al., 1997, J. Biol. Chem. 272, 33435-33433), SRC-1 (Onate et al., 1995, Science 270, 1354-1357), , TIF2 (GRIP-2 ; Chakravarti et al., 1996, Nature, 383, 99-103), SRA (Lanz et al., 1999, Cell, 97, 17-27), AIB-1 (Anzick et al., 1997, Science, 277, 965-968), TRAP220/DRIP205 (i.e. PBP ; Zhu et al., 1997, J. Biol. Chem. 272, 25500-25506 ; Rachez et al., 1999, Nature, 398, 824-828), PGC-1 (Puigserver et al., 1998, Cell 92, 829-839), PRIP (Zhu et al., 2000, J Biol Chem 275, 13510-13516), PGC-2 (Castillo et al., 1999, Embo J , 18, 3676-3687), ARA70 (Heinlein et al., 1999, J Biol Chem 274, 16147-16152), RIP140 (Treuter et al., 1998, Mol Endocrinol 12, 864-881), enhance their transcriptional activity, whereas SMRT (Lavinsky et al., 1998, Proc. Natl. Acad. Sci. USA 95, 2920-2925) and N-CoR (Dowell et al., J Biol Chem 274, 15901-15907) repress it. Additionally, it has been shown that members of the PPAR-gamma cofactor family (e.g. the 160-kDa protein (SRC-1/TIF2/AIB-1), CBP/p300 or TRAP220/DRIP205) interact directly with PPAR-gamma and potentiate nuclear receptor transactivation function in a ligand-dependent fashion leading to biological action or side effects that can differ according to the ligand

used (Adams et al., 1997, J. Clin. Invest., 100, 3149-3153). Kodera et al. (2000, J Biol Chem., 275, 33201- 33204) have examined whether interactions between PPAR-gamma and known cofactors were induced to the same extent by different classes of PPAR-gamma ligands (natural and synthetic) and concluded that the overall structure of PPAR-gamma and cofactors complexes may be different according to the ligands involved, resulting in the activation of a particular set of target gene promoters that exert different biological actions.

Adipose tissue is a tissue highly relevant to metabolism and is implicated in a variety of important disorders, such as obesity, insulin resistance, type 2 diabetes mellitus, hyperlipidemia, hypertension, atherosclerosis, cachexia, lipo-atrophy, lipo-dystrophy, chronic inflammatory disorders and cancer. Said tissue expresses a number of important nuclear receptors, including, but not limited to, PPAR-gamma, PPAR-beta or delta, PPAR- alpha, thyroid hormone receptor (TR), retinoid acid receptors (RARs), estrogen receptor alpha (ERα), ERβ, retinoid X receptors (RXRs), Liver X receptor (LXRα, LXRβ), Rev-Erb alpha, Estrogen-related receptor alpha (ERRα), glucocorticoid receptor (GR), progesterone receptor (PR), mineralocorticoid receptor (MR) which are drug targets.

The inventors have now undertaken a systematic screen of a yeast two hybrid cDNA library made from human white adipose tissue and have isolated a number of new nuclear receptor cofactors. Moreover, the inventors have shown that these cofactors are PPAR-gamma and/or interacting proteins which can regulate PPAR-gamma activity.

In first aspect, the present invention concerns human polypeptides comprising the N-terminal amino acid sequence set forth in SEQ ID NO:10 (i.e. Met-Ala-Ala-Asp-Glu-Val-Ala-Gly-Gly-Ala-Arg-Lys-Ala-Thr-Lys-Ser-Lys-Leu-Phe-Glu-Phe-Leu-Val-His-Gly-Val), or a conservatively substituted variant thereof. According to a preferred embodiment, said polypeptides are nuclear receptor cofactors.

The term "nuclear receptor" designates a specific molecule, e.g., protein, within a cell that recognizes and binds to other specific molecules, and more specifically to targeted nucleic acid sequences. In preferred embodiment, the nuclear receptor of the invention is a transcription factor, and more preferably it belongs to the nuclear hormone receptor superfamily. More preferably, the nuclear receptor represents amember of the superfamily of structurally related ligand-activated transcription factors, which control the expression of target genes and thus regulate diverse biological activities like growth, development, and homeostasis. According to a special embodiment, the nuclear receptor of the invention is the peroxisome proliferator-activated receptor (PPAR).

The terms "nuclear receptor cofactor" designates a molecule, e.g., protein, within the cell that recognizes and binds (i.e. interacts) to the nuclear receptor, said interaction results in the recruitment of other cofactors or other compounds (e.g. ligands) by the nuclear receptor, and facilitates the recognition and binding of the nuclear receptor to its target molecule (e.g. targeted nucleic acid sequences), and/or in modulation of transcriptional effect of the nuclear receptor. The cofactors include both so called coactivators and corepressors. Alternatively, the binding of the nuclear receptor cofactor is modulated (inhibited or enhanced) by compounds, such as ligands.

According to particular embodiment, the polypeptide of the invention consists in all or part of the polypeptide set forth in SEQ ID No 12. Partial polypeptide of interest is for example, a polypeptide comprising residues 1 to 193 of the polypeptide set forth in SEQ ID No 12. Another polypeptide of interest according to the present invention consists of all or part of the polypeptide set forth in SEQ ID No 13.

According to particular embodiment, the polypeptide of the invention is a PPAR and / or a RXR cofactor. In preferred embodiment, it is selected from a group consisting of PPAR-gamma cofactors and RXR-alpha cofactors.

The term "PPAR" designates the transcription factors peroxisome proliferator-activated receptors as defined above. The term "RXR" designates members of the retinoid X receptor subfamily including RXR-alpha, RXR-beta and RXR-gamma (De

Luca, 1991, FASEB J., 5, 2924-2933). RXR has been found to heterodimerize with other nuclear receptors (e.g. PPAR-gamma) to form active transcriptional complexes, which influence the activity of a variety of gene pathways important in growth and differentiation. RXR ligands include naturally occurring retinoic acid and synthetic derivatives that have been termed retinoids or rexinoids.

According to particular embodiment, the polypeptides of the invention are recombinant polypeptides. The terms "recombinant polypeptides" relate to any molecule having a polypeptidic chain that can be produced by genetic engineering, through transcription and translation, of a corresponding DNA sequence under the control of appropriate gene regulatory elements within an efficient cellular host. Consequently, the expression "recombinant polypeptides" such as those used herein does not exclude the possibility for the polypeptides to comprise of other groups, such as glycosylated groups. The term "recombinant" indeed means that the polypeptide has been produced by genetic engineering, particularly because it results from the expression in a cellular host of the corresponding nucleic acid sequences which have previously been introduced into an expression vector used in said host. Nevertheless, it must be understood that the polypeptides of the invention can be produced by a different process, for instance by classical chemical synthesis according to methods used in the protein synthesis or by proteolytic cleavage of larger molecules.

According to special embodiments, the free reactive functions which are present in some of the amino acids of the polypeptides of the invention, particularly the free carboxyl groups which are carried by the groups Glu and Asp or by the C-terminal amino acid on one hand and/or the free NH_2 groups carried by the N-terminal amino acid or by amino acids inside the peptidic chain, for instance Lys, on the other hand, can be modified in so far as this modification does not alter the above mentioned properties of the polypeptide. The molecules which are thus modified are naturally part of the invention. The above mentioned carboxyl groups can be acylated or esterified. Other modifications are also part of the invention.

According to specific applications of the invention, it would be desirable to modify or label the polypeptide in order to allow their detection or in order to visualize their interaction with other compounds (e.g. heterologous proteins). Methods for modifications/labeling polypeptides are well known by those skilled in the art. As an example, please refer to Chapter 18 of Sambrook and Russell (Molecular Cloning ; Third Edition, 2001, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York). The polypeptide may be attached to a detectable label.

The invention also relates to the amino acid sequences constituted by the above mentioned polypeptides and a protein or an heterologous sequence with respect to said polypeptide, said protein or heterologous sequence comprising for instance from about 1 to about 1100 amino acids. These amino acid sequences will be called fusion proteins or chimeric proteins. Particularly, the amine or carboxyl functions of both terminal amino acids can be themselves involved in the bond with other amino acids. Examples of these "protein or an heterologous sequences" are: (i) epitopes (see Chapter 17, pages 17.90-17.94 of Sambrook and Russell (Molecular Cloning ; Third Edition, 2001, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York), and more preferably His-6 epitope recognized by monoclonal antibodies Mabs 6-His, 6xHis and HIS-11, or an epitope selected from the group consisting in substance P, Human c-Myc protein, colicin A protein, influenza virus hemagglutining, SV40 T antigen, FLAG sequence, T7-Tag, AU epitopes, HPOL, Btag, 3b3, IRS, or polypeptide sequences derived there from ; (ii) glutathione-S-transferase or GST protein (Smith and Johnson, 1988, Gene, 67, 31-40).

Furthermore, any peptidic sequences resulting from the modification by substitution and/or by addition and/or by deletion of one or several amino acids of the polypeptides according to the invention are part of the invention in so far as this modification does not alter the properties and characteristics of said polypeptides.

According to a specific embodiment, the invention concerns a chimeric protein comprising a polypeptide as described above fused to a heterologous polypeptide, and more specifically to glutathione-S-transferase (GST) or His-6 epitope.

According to another aspect, the invention concerns an isolated, enriched, or purified nucleic acid molecule encoding the polypeptides of the invention, or able to hybridize with said nucleic acid molecule encoding the polypeptides of the invention. The term "nucleic acid molecule" describes a polymer of deoxyribonucleotides (DNA) or ribonucleotides (RNA). The nucleic acid molecule may be isolated from a natural source by cDNA cloning (e.g. by PCR) or subtractive hybridization or synthesized manually. The nucleic acid molecule may be synthesized manually by the triester synthetic method or by using an automated DNA synthesizer.

The polypeptides of the invention can be encoded by a full-length nucleic acid sequence or any portion of the full-length nucleic acid sequence. In preferred embodiments the isolated nucleic acid comprises, consists essentially of, or consists of a nucleic acid sequence set forth in SEQ ID NO:1 or in SEQ ID NO:2, a nucleic acid sequence that hybridizes to the nucleic acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2, or functional derivatives of either. The nucleic acid may be isolated from a natural source by cDNA cloning or subtractive hybridization.

In reference to nucleic acid, "isolated" is meant a polymer of 14, 17, 21 or more nucleotides conjugated to each other, including DNA or RNA that is isolated from a natural source or that is synthesized. The isolated nucleic acid of the present invention is unique in the sense that it is not found in a pure or separated state in nature. Use of the term "isolated" indicates that a naturally occurring sequence has been removed from its normal cellular (i.e. chromosomal) environment. Thus, the sequence may be in a cell-free solution or placed in a different cellular environment. The term does not imply that the sequence is the only nucleotide sequence present, but that it is essentially free (about 90-95% pure at least) of nucleotide material naturally associated with it and thus is meant to be distinguished from isolated chromosomes.

The term "enriched" in reference to nucleic acid is meant to indicate that the specific DNA or RNA sequence constitutes a significantly higher fraction (2-5 fold) of the total DNA or RNA present in the cells or solution of interest than in normal or

diseased cells or in the cells from which the sequence was taken. This could be caused by a person by preferential reducing in the amount of other DNA or RNA present, or by a preferential increase in the amount of the specific DNA or RNA sequence, or by a combination of the two. However, it should be noted that "enriched" does not imply that there are no other DNA or RNA sequences present, just that the relative amount of the sequence of interest has been significantly increased.

The term "significant" here is used to indicate that the level of increase is useful to the person making such an increase, and generally means an increase relative to other nucleic acids of at least 2 fold, more preferably at least 5 to 10 fold or even more. However, the term does not imply that there is no DNA or RNA from other sources. The other source DNA may, for example, comprise DNA from a yeast or bacterial genome, or a cloning vector such as pUC19 or phage.

It could also be advantageous for some purposes that a nucleotide sequence is present in purified form. The term "purified" in reference to nucleic acid does not require absolute purity (such as a homogeneous preparation); instead, it represents an indication that the sequence is relatively purer than in the natural environment (compared to the natural level this level should be at least 2-5 fold greater, e.g., in terms of mg/mL). Individual clones isolated from a cDNA library may be purified to electrophoretic homogeneity. The claimed DNA molecules obtained from these clones can be obtained directly from total DNA or from total RNA. The cDNA clones are not naturally occurring, but rather are preferably obtained via manipulation of a partially purified naturally occurring substance (messenger RNA). The construction of a cDNA library from mRNA involves the creation of a synthetic substance (cDNA) and pure individual cDNA clones can be isolated from the synthetic library by clonal selection of the cells carrying the cDNA library. Thus, the process which includes the construction of a cDNA library from mRNA and isolation of distinct cDNA clones yields an approximately 10^6 fold purification of the native message.

The terms "hybridize or hybridization" refer to a method of interacting (binding/attaching) a nucleic acid sequence with a DNA or RNA molecule in solution or on a solid support, such as cellulose or nitrocellulose. If a nucleic acid sequence binds to the DNA or RNA molecule with high affinity, it is said to "hybridize" to the DNA or RNA molecule. The strength of the interaction between the probing sequence and its target can be assessed by varying the stringency of the hybridization conditions. Under highly stringent hybrydization conditions only highly complementary nucleic acid sequences hybridize. Preferably, such conditions prevent hybridization of nucleic acids having one or two mismatches out of 20 contiguous nucleotides. Various low or high stringency hybridization conditions may be used depending upon the specificity and selectivity desired. Stringency is controlled by varying salt or denaturant concentrations. Examples of hybridization conditions are shown in the examples below. High stringent conditions may mean conditions that are at least as stringent as the following: hybridization in 50% formamide, 5xSSC, 50 mM NaH_3 PO_4, pH 6.8, 0.5% SDS, 0.1 mg/mL sonicated salmon sperm DNA, and 5xDenhart solution at 42° C overnight; washing with 2x SSC, 0.1% SDS at 45° C; and washing with 0.2.x.SSC, 0.1% SDS at 45° C. Those skilled in the art will recognize how such conditions can be modified to vary specificity and selectivity.

According to another embodiment, the present invention concerns a method of screening for compounds that modulate the interaction of at least one nuclear receptor with at least one nuclear receptor cofactor of the invention, the method comprising :

(i) incubating a reaction mixture comprising :

- at least one nuclear receptor, or fragments thereof,
- at least one potential binding modulator and,
- at least one nuclear receptor cofactor or at least one chimeric protein of the invention, or fragments thereof, and

(ii) determining whether the binding of said nuclear receptor cofactor, or of said chimeric protein, to said nuclear receptor is increased or decreased in comparison to an assay which lacks the potential binding modulator.

According to one embodiment, the reaction mixture of step (i) comprises a fragment of the nuclear receptor, said fragment including the C-terminal domain of said receptor, and in more specific embodiment said fragment of the nuclear receptor comprises the E domain (i.e. ligand binding LBD).

In another embodiment, the nuclear receptor is PPAR, and more preferably it is PPAR-gamma. In special embodiments, it is selected from the group of PPAR-gamma1 and PPAR-gamma2. In special embodiments, the fragment of PPAR implemented in step (i) of the above screening method is defined in PPAR-gamma and contains either the b exon and the AB domains (b-AB), the AB domain (AB), the DE domain (DE) of PPAR-gamma and/or the E domain in still another embodiment it is containing the DE domain (DE) (Greene et al., 1995, Gene Expression, 4, 281-299). In one embodiment, said PPAR-gamma protein or fragment is of human origin (see also reference P37231 of NCBI data base). According to preferred embodiments, the fragment used in step (i) contains residues 229 to 505 of human PPAR-gamma. Alternatively, it is defined from PPAR-gamma of rat (O88275), from pig (O62807), chicken (AAL85323), mouse (P37238), bovin (O18971). These NCBI references and their contents are incorporated herein by reference.

In another embodiment, the nuclear receptor is RXR, and more specifically RXR alpha. In special embodiments, the fragment of RXR implemented in step (i) of the above screening method is the RXR-alpha protein and contains the E domain (i.e. residues 225 to 462 of human RXR-alpha).

According to a special embodiment, the nuclear receptor or related fragment implemented in step (i) is modified or labeled in order to allow its detection or in order to visualize its interaction with other compounds. Methods for labeling and modifying polypeptides have been disclosed above (see labeling and modification of the

polypeptides of the invention). Similarly, the nuclear receptor implemented in step (i) can be a recombinant nuclear receptor or it can be produced, for instance, by classical chemical synthesis according to methods used in the protein synthesis or by proteolytic cleavage of larger molecules.

Methods and conditions for screening compounds able to interact with nuclear receptors (e.g. ligands) are widely disclosed in the art : for example, Glickman et al., 2002, J. Biomolecular Screening, 7, 3-10 ; Le Douarin et al., (2001, Methods Mol Biol, 176, 227-48) have disclosed an *in vitro* screening test using the yeast two-hybrid system that is based on the ligand-dependent interaction of two proteins, a hormone receptor and a coactivator ; Zhou et al., (2001, Methods, 25, 54-61) have disclosed a homogeneous time-resolved fluorescence (HTRF) energy transfer technology which is sensitive, homogeneous, and nonradioactive ; Beaudet et al, (2001,Genome Res, 11, 600-8) have disclosed the AlphaScreen™ technology (Packard BioScience) which allows the development of high-throughput homogeneous proximity assays. The full content of these papers is incorporated herein by reference.

The terms "modulate the interaction" or "modulate the binding" are synonymous and can be used interchangeably. This modulating effect is defined by reference to the natural situation, i.e. the natural binding observed when the tested nuclear receptor cofactor, or related chimeric protein, is contacted with the tested nuclear receptor. Modulation refers to an increase or a decrease of said binding when the "potential binding modulator" is present in the mixture (i). Generally, an increase of said interaction/binding is assimilated to an enhancement and relates to compounds named activators, agonists or partial agonists. On the contrary, a decrease of said interaction/binding is assimilated to an inhibition and relates to compounds named inhibitors or antagonists.

According to specific embodiment, the method of the invention is a screening method for compounds that enhance the interaction of said nuclear receptor with said nuclear receptor cofactor.

According to another embodiment, the present invention concerns a method of screening for compounds that modulate the interaction of at least one nuclear receptor with a specific selection of nuclear receptor cofactor, the method comprising the following steps :

(a) testing the modulating effect of at least one potential binding modulator on the interaction of at least one nuclear receptor with at least one nuclear receptor cofactor of the invention by incubating a reaction mixture comprising :

- at least one nuclear receptor, or fragments thereof,
- at least one potential binding modulator and,
- at least one nuclear receptor cofactor or at least one chimeric protein of the invention, or fragments thereof,

(b) testing the modulating effect of the same potential binding modulator on the interaction of the same nuclear receptor with at least one nuclear receptor cofactor different from the one tested in (a) by incubating a reaction mixture comprising :

- the same nuclear receptor, or fragments thereof, as in step (a)
- the same potential binding modulator as in step (a) and,
- at least one nuclear receptor cofactor or at least one chimeric protein, or fragments thereof, said nuclear receptor cofactor/chimeric protein being different from the one implemented in step (a),

(c) determining for test (a) and test (b) whether the binding of the respective nuclear receptor cofactor, or chimeric protein, to the respective nuclear receptor is increased or decreased in comparison to an assay which lacks the potential binding modulator, and

(d) comparing the modulating effects of the tested potential binding modulator observed in test (a) and test (b).

According to special embodiments, the test (b) is individually and independently reproduced using different nuclear receptors cofactors. In preferred embodiments, said nuclear receptors implemented in the test (b) are cofactors previously identified and characterized. In preferred embodiments, the nuclear receptor cofactor implemented in the test (b) is selected from the group consisting of p300/CBP (Dowell et al., 1997, *supra*), SRC-1 (Onate et al., 1995, *supra*), TIF2 (GRIP-2 ; Chakravarti et al., 1996, *supra*), SRA (Lanz et al., 1999, *supra*), AIB-1 (Anzick et al., 1997, *supra*), TRAP220/DRIP205 (PBP ; Zhu et al., 1997, *supra* , Rachez et al., 1999, *supra*), PGC-1 (Puigserver et al., 1998, *supra*), PRIP (Zhu et al., 2000, *supra*), PGC-2 (Castillo et al., 1999, *supra*), ARA70 (Heinlein et al., 1999, *supra*), RIP140 (Treuter et al., 1998, *supra*), SMRT (Lavinsky et al., 1998, *supra*) and N-CoR (Dowell et al., *supra*).

According to another embodiment, the method of the invention further comprises step (iii) consisting of assessing the effect of the modulation of the binding of said nuclear receptor cofactor to said nuclear receptor as determined in step (ii) on the transactivator activity of said nuclear receptor.

Methods for analyzing the modulation of transactivator activity of said nuclear receptor can be examined in a reconstituted system in cell culture (for example see US 5,668,175). Such a system was used to evaluate the compounds of this invention for their interaction with the nuclear receptor. The system for reconstituting ligand-dependent transcriptional control has been developed by Evans et al., 1988, Science, 240, 889-95 and has been termed "co-transfection" or "cis-trans" assay. This assay is described in more detail in U.S. 4,981,784 and US 5,071,773, which are incorporated herein by reference. Also see Heyman et al., Cell, 68: 397-406 (1992). The co-transfection assay provides a method to evaluate the ability of a compound to modulate the transcriptional response initiated by a nuclear receptor. The co-transfection assay is a functional, rapid assay that monitors hormone or ligand activity, is a good predictor of the *in vivo* activity, and can be used to quantitate the pharmacological potency and utility of such ligands in treating various disease states (Berger, et al., 1992, J. Steroid

Biochem Molec. Biol., 41: 733-38). Briefly, the co-transfection assay involves the introduction of various plasmids by transient transfection into a mammalian cell : a plasmid which contains a nuclear receptor receptor cDNA (e.g. PPAR gamma or RXR alpha) and directs constitutive expression of the encoded receptor ; a plasmid which contains a sequence encoding one cofactor polypeptide of the invention and directs constitutive expression of the encoded cofactor and a plasmid which contains a cDNA that encodes for a readily quantifiable protein, e.g., firefly luciferase or chloramphenicol acetyl transferase (CAT), alkaline phosphatase (SPAP or SEAP), under control of a promoter containing a PPAR response element (PPRE), which confers dependence on the transcription of the reporter. This assay can be used to accurately measure efficacy and potency of potential binding modulator.

According to another embodiment, the method of the invention further comprises step (iv) consisting of determining the cell and/or tissue specificity :

- of the modulation of the binding of said nuclear receptor cofactor to said nuclear receptor as determined in step (ii), and/or
- of the effect of said modulation on the transactivator activity of said nuclear receptor as determined in step (iii).

Cell and/or tissue specificity can be assessed by cell/tissue-profiling . Said profiling consists of the analysis of the modulation of the binding and/or of transactivator activity of said nuclear receptor in a reconstituted system in many cell culture, wherein said cells are originated from various tissue.

The present invention further concerns a method for identifying a compound that modulates at least one nuclear receptor function comprising a screening method as disclosed above. According to preferred embodiment, said method is intended for identifying a compound that modulates the PPAR-gamma function and/or RXR function.

In another aspect, the present invention concerns a compound that modulates the interaction of at least one nuclear receptor with at least one nuclear receptor cofactor of the invention. According to a special embodiment, said compound enhances said interaction. According to another embodiment, said compound further modulates function of at least one nuclear receptor, and preferably said compound enhances function of at least one nuclear receptor.

In a preferred embodiment, said compounds are identified by a screening or identifying method of the present invention. In a special embodiment, it is a PPAR-gamma receptor modulator and/or a RXR-alpha receptor modulator.

According to one embodiment, the compound of the invention is a modulator selected from the group consisting of nuclear receptor agonists and nuclear receptor partial agonists.

According to the present invention, the term " nuclear receptor agonist" means nuclear receptor ligand which when combined with its respective nuclear receptor increases a reaction typical for the said receptor, e.g., transcriptional regulation activity. In one embodiment, said agonist is a PPAR–gamma agonist, i.e. a PPAR ligand which potentiates, induces or otherwise enhances the transcriptional activity of a PPAR-gamma receptor, e.g., such as by mimicking a natural ligand for the receptor or as measured by an assay known to one skilled in the art, including, but not limited to, the "co-transfection" or "cis-trans" assays described or disclosed in US 4,981,784, US 5,071,773 or Lehmann et al., 1995, J. Biol. Chem., 270, 12953-12956, which are incorporated by reference herein. More specifically, it has been shown that activation of PPAR activity (i.e. activation of PPAR targeted genes transcription) by PPAR agonist results actually from promoted recruitment of coactivator proteins (e.g. the polypeptide of the invention) to the PPAR receptor by said PPAR agonists.

"Nuclear receptor antagonists" have been described elsewhere (see WO 01/17994). The term "PPAR-gamma antagonist" designates a PPAR-gamma ligand that gives greater than 50% inhibition of transactivation achieved by 100 nM rosiglitazone

when tested in the cell-based reporter assay as described in WO 01/17994. As general definition, "PPAR antagonist" designates a PPAR ligand which can inhibit the activity of a corresponding PPAR agonist.

The present invention further concerns a composition comprising at least one compound of the invention as disclosed above and a pharmaceutically acceptable carrier or diluent. These pharmaceutical compositions may be prepared by conventional techniques, e.g. as described in Remington, 1995, The Science and Practise of Pharmacy, 19.sup.th Ed. They may appear in conventional forms, for example capsules, tablets, aerosols, solutions, suspensions or topical applications.

Typical compositions of the present invention are associated with a pharmaceutically acceptable excipient which may be a carrier or a diluent or be diluted by a carrier, or enclosed within a carrier which can be in the form of a capsule, sachet, paper or other container. In making the combination products, conventional techniques for the preparation of pharmaceutical compositions may be used. For example, the active compounds will usually be mixed with a carrier or a diluent, or diluted by a carrier or a diluent, or enclosed within a carrier or a diluent which may be in the form of a ampoule, capsule, sachet, paper, or other container. When the carrier serves as a diluent, it may be solid, semi-solid, or liquid material which acts as a vehicle, excipient, or medium for the active compound. The active compounds can be adsorbed on a granular solid container for example in a sachet. Some examples of suitable carriers or diluents are water, salt solutions, alcohols, polyethylene glycols, polyhydroxyethoxylated castor oil, peanut oil, olive oil, gelatine, lactose, terra alba, sucrose, cyclodextrin, amylose, magnesium stearate, talc, gelatin, agar, pectin, acacia, stearic acid or lower alkyl ethers of cellulose, silicic acid, fatty acids, fatty acid amines, fatty acid monoglycerides and diglycerides, pentaerythritol fatty acid esters, polyoxyethylene, hydroxymethylcellulose and polyvinylpyrrolidone. Similarly, the carrier or diluent may include any sustained release material known in the art, such as glyceryl monostearate or glyceryl distearate, alone or mixed with a wax. The formulations may also include wetting agents, emulsifying and

suspending agents, preserving agents, sweetening agents or flavouring agents. The formulations of the invention may be formulated so as to provide quick, sustained, or delayed release of the active ingredient after administration to the patient by employing procedures well known in the art. In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811. The pharmaceutical compositions of the invention can be sterilized and mixed, if desired, with auxiliary agents, emulsifiers, salt for influencing osmotic pressure, buffers and/or colouring substances and the like, which do not deleteriously react with the active compounds.

The invention also concerns methods for treating and/or preventing diseases or pathologic conditions associated with cell types that express PPAR receptors comprising administering to the patient at least one compound of or a composition of above disclosed.

Similarly, the invention refers to the use of at least one compound of the invention for the preparation of a pharmaceutical composition.

It further concerns the use of at least one compound of the invention for the preparation of a pharmaceutical composition for treating and/or preventing diseases or pathologic conditions associated with cell types that express PPAR receptors.

Alternatively, the composition of the present invention further comprises a natural or synthetic PPAR and/or RXR agonist or antagonist.

Naturally occurring ligands that modulate the activity of PPAR, preferably the PPAR-gamma, including but are not limited to, fatty acids such as arachidonic acid derivatives or metabolites such as eicosanoids (e.g. various isomeric forms of 8-hydroxytetraenoic acid) and cyclopentenone prostaglandins (e.g. prostaglandins in the J and A series and their metabolites), long-chain fatty acids and their derivatives, e.g. 9- and 13-cis-hydroxyoctadecadienoic acid (HODE) (Nagy et al., 1998, Cell, 17, 93, 229-240 ; Chinetti et al., 2001, Z Kardiol, 90 Suppl 3, 125-32). Diterpene acids and auronols (e.g. pseudolaric acids A and B) isolated from Pseudolarix kaempferi (Pan et al., 1990, Planta Med, 56, 383-385; Li et al., 1999, J Nat Prod, 62, 767-769) have also been shown to activate PPAR-gamma and are expected to be useful in the practice of this invention. In one embodiment, said natural PPAR ligand is a prostaglandin J2 or delta-12-prostaglandin J2 (PGJ2) metabolite, and more particularly it is 15-deoxy-delta-12,14-prostaglandin J2 [15-deoxy-Delta(12,14)-PGJ(2) or 15d-PGJ2].

Synthetic ligands that modulate the activity of PPAR are for example antidyslipidemic fibrates (e.g. clofibrate, fenofibrate, benzofibrate, ciprofibrate, gemfibrozil), thiazolidine derivatives (e.g. thiazolidinediones), oxazolidine derivatives (e.g. oxazolidinediones), alpha-alkylthio, alpha-alkoxy and carboxylic acid derivatives of thiazolidines and oxazolidines (Hulin et al. 1996, J Med Chem., 39, 3897-3907), N-2-L-tyrosine derivatives (e.g. N-(2-Benzoylphenyl)-L-tyrosine ; Henke et al., 1998, J Med Chem, 41, 5020-5036), FMOC-L-Leucine (WO0200611), phenyl acetic acid derivatives (Berger et al., 1999, J. Biol. Chem., 274, 6718-6725) and indole-thiazolidinedione derivatives (Lohray et al., 1998, J Med Chem, 41, 1619-1630).

Compounds disclosed or described in the following articles, patents and patent applications which have RXR agonist activity are incorporated by reference herein: US 5,399,586 and 5,466,861, WO96/05165, WO94/15901, WO93/11755, WO94/15902, WO93/21146, Boehm, et al. 1994, J. Med. Chem. 38, 3146-3155, Boehm, et al. 1994, J.

Med. Chem. 37, 2930-2941, Antras et al., 1991, J. Biol. Chem. 1266, 1157-1161. RXR specific agonists include, but are not limited to, 9-cis-retinoic acid, 4-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)-ethenyl)benzoic acid (3-methyl-TTNEB; LGD 1069), LG 100268 (i.e. 2-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)-cyclopropyl]-pyridine-5-carboxylic acid), 4-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthy)-2-carbonyl]-benzoic acid, ((E)-2-(2-((5,6,7,8-tetra-hydro-3,5,5,8,8-pentamethyl-2-naphthyl)propen-1- yl)-4-thiophenecarboxylic acid) (AGN 191701), 2-(5,6,7,8-tetra-hydro-5,5,8,8-tetramethyl-2-naphthyl)-2-(carboxyphenyl)-1 ,3-dioxolane (SR 11237), 4-(5H-2,3-(2,5-dimethyl-2,5-hemano)-5-methyl-dibenzo(b,e)(1,4)diazepin-11-yl)-benzoic acid (HX600) or thiadiazepin analogs thereof, 3,7,11,15-tetramethyl hexadecanoic acid (phytanic acid), 6-(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopropyl) nicotinic acid, ALRT 1057 (i.e. 9-cis retinoic acid, 2-(4-carboxyphenyl)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalen yl)-1,3-dithiane (SR11203), 4-(2-methyl)-1-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)propenyl)benzoic acid (SR11217), and the like or a pharmaceutically acceptable salt thereof.

Likewise, the compositions of the present invention can further comprise additional agents. Examples of such additional agents are insulin, insulin derivative, insulin secretagogue, insulin sensitizer, or insulin mimetic ; other examples are mitotic inhibitors, alkylating agents, antimetabolites, nucleic acid intercalating agents, topoisomerase inhibitors, agents which promote apoptosis, or agents which increase immune responses to tumors (e.g cytokine chosen from alpha-, beta- and gamma-interferon, interleukins, and in particular IL-2, IL-4, IL-6, IL-10 or IL-12, tumour necrosis factors (TNFs) and colony stimulating factors (for example GM-CSF, C-CSF and M-CSF). Literature provides to the skilled man with numerous examples of such additional agents.

The compounds and compositions of the present invention are specially adapted to cure, improve or prevent one or more symptoms of diseases or pathologic conditions associated with cells types that express PPAR nuclear receptors.

"Diseases or pathologic conditions associated with cells types that express PPAR nuclear receptors" means diseases or pathologic conditions wherein the observed disorder is associated initially with the deregulation, disturbance, hypersensitivity, or malfunctioning of cells expressing PPAR nuclear receptors, preferably PPAR-gamma receptor, or more specifically in which the disease or pathologic conditions is caused by one or more genes that are under the transcription control of PPARs and preferably of PPAR-gamma, or said disease or pathological condition causing genes are posttranlationally modified in response to PPARs. Examples of these cells are those from liver, skeletal muscle, kidney, heart, CNS, adipose tissues, intestine, or cells of the monocyte lineage. In preferred embodiment, said cell type is an adipocyte or pre-adipocyte. Another example is a PPAR-responsive hyperproliferative cell. Examples of diseases or pathologic conditions associated with cells types that express PPAR nuclear receptors are those associated with impaired metabolism of glucose, cholesterol or triglycerides. More specifically, it is insulin resistance, type 2 diabetes, impaired glucose tolerance, dyslipidemia, hypercholesterolemia, hypertriglycidemia, disorders related to the metabolic disease Syndrome X including hypertension, obesity, hyperglycaemia, atherosclerosis, thrombosis, hyperlipidemia, coronary artery disease and other cardiovascular disorders ; renal diseases including glomerulonephritis, glomerulosclerosis, nephrotic syndrome, hypertensive nephrosclerosis ; neurologic diseases or dementia ; inflammatory diseases such as cutaneous disorders (including acne vulgaris, psoriasis, cutaneous disorders with barrier dysfunction, cutaneous effects of aging, poor wound healing), diabetic complications, polycystic ovarian syndrome (PCOS) and bone loss, e.g. osteoporosis ; gastrointestinal diseases ; viral, proliferative cells or tumoral diseases, such as cancers.

In one embodiment, disease or pathologic condition according to the invention is obesity. Obesity is a disease that had become highly prevalent in affluent societies and in the developing world and which is a major cause of morbidity and mortality. It is characterized by a body mass index superior to 25. Thus there is a strong need for efficient therapy to treat this disease which has been identified as a leading cause of coronary heart disease, type 2 diabetes, stroke, hyperlipidemia, gout, osteoarthritis, reduced fertility and many other psychological and social problems.

In another embodiment, disease or pathologic condition according to the invention is diabetes or insulin resistance. Insulin resistance is manifested by the diminished ability of insulin to exert its biological action across a broad range of concentrations. During early stages of insulin resistance, the body secretes abnormally high amounts of insulin to compensate for this defect. Among the developed countries, diabetes mellitus is a common problem and is associated with a variety of abnormalities including, but not limited to, obesity, hypertension, hyperlipidemia and renal complications. It is now increasingly being recognized that insulin resistance and hyperinsulinemia contribute significantly to obesity, hypertension, atherosclerosis and type 2 diabetes mellitus. The association of insulin resistance with obesity, hypertension and angina pectoris has been described as a syndrome (Syndrome-X) in which insulin resistance plays the central role. The term "diabetes" refers to all variant forms of diabetes mellitus (DM), including type 1 DM, type 2 DM, gestational diabetes, juvenile diabetes, etc.

In another embodiment, disease or pathologic condition according to the invention is hyperlipidemia. Hyperlipidemia is considered the primary cause of cardiovascular and other peripheral vascular diseases. An increased risk of cardiovascular disease is correlated with elevated plasma levels of LDL (Low Density Lipoprotein) and VLDL (Very Low Density Lipoprotein) as seen in hyperlipidemia. Numerous studies have shown that lowering of plasma triglycerides and total

cholesterol, in particular LDL and VLDL and increasing HDL cholesterol leads to a significant reduction of cardiac events.

In yet another embodiment, "diseases or pathologic conditions associated with cells types that express PPAR nuclear receptors" also include cellular proliferation, growth, differentiation, or migration disorders. As used herein, a "cellular proliferation, growth, differentiation, or cell migration disorders" is a disorder in which a cell increases in number, size or content, in which a cell develops a specialized set of characteristics which differ from that of other cells, or in which a cell moves closer to or further from a particular location or stimulus. The PPAR molecules of the present invention are involved in signal transduction mechanisms, which are known to be involved in cellular growth, differentiation, and migration processes. Thus, the PPAR molecules may modulate cellular growth, differentiation, or migration, and may play a role in disorders characterized by aberrantly regulated growth, differentiation, or migration. Such disorders include cancer, e.g., carcinomas, sarcomas, leukemias, and lymphomas; tumor angiogenesis and metastasis; skeletal dysplasia; hepatic disorders; and hematopoietic and/or myeloproliferative disorders. Exemplary disorders include, but not limited to, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, and retinoblastoma.

In other embodiments, the disease or pathologic condition according to the invention is a disorder characterized by aberrant cell growth of PPAR-responsive cells such as hyperplastic or neoplastic disorders arising in adipose tissue, such as adipose cell tumors, e.g., lipomas, fibrolipomas, lipoblastomas, lipomatosis, hibemomas, hemangiomas and/or liposarcomas.

In still other embodiments, the disease or pathologic condition according to the invention is a disorder characterized by aberrant cell growth of PPAR-responsive cells such as hyperplastic or neoplastic disorders of the hematopoietic system, e.g., leukemic cancers.

In another embodiment, disease or pathologic condition according to the invention is an inflammatory disease including, but not limited to, T-lymphocyte activation and other T-lymphocyte-related disorders ; inflammatory cytokine (e.g. TNF-alpha, interleukin (IL)-1-alpha, IL-1-beta, IL-2, IL-6) production ; activation of nuclear factors that promote transcription of genes encoding inflammatory cytokines. Examples of these nuclear transcription factors include but are not restricted to, nuclear factor-kappaB (NF-kappaB), activated protein-1 (AP-1), nuclear factor of activated T cells (NFAT).

Other examples of disease or pathologic condition according to the invention are chronic viral infections (e.g. HIV, CMV, HSV, HBV, HCV infections), neurodegenerative diseases (e.g. Alzheimer's disease, multiple sclerosis, Parkinson's disease), cardiovascular disease (e.g. atherosclerosis, atherogenesis, vascular restenosis, congestive heart failure), diseases or conditions involving hypoxemia and hypoxic stress (stroke, vascular occlusive disease, MI, atherosclerosis, retinitis, retinal vein occlusion, hypoxic retinopathy, macular degeneration).

According to the present invention, the term "patient" means a mammal, e.g., a primate, e.g., a human.

According to another aspect, the invention provides a method of treating and/or preventing nuclear receptors PPAR mediated diseases or conditions in an patient, comprising the step of administering to said individual a pharmacologically effective dose of a compound or composition of the invention. As used herein, "treatment" of an individual includes the application or administration of a compound or product of the invention to an individual, or application or administration of a compound or product of the invention to a cell or tissue from an individual, who has a diseases or disorder, has a symptom of a disease or disorder, or is at risk of (or susceptible to) a disease or disorder, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect the disease or disorder, the symptom of the disease or disorder, or the risk of (or susceptibility to) the disease or disorder.

Alternatively, the present invention concerns a method of treating and/or preventing diseases or conditions in an patient, comprising the step of administering to said individual a pharmacologically effective dose of a compound or composition of the invention said administration resulting in improving the clinical status of said patient.

By "pharmaceutically effective dose" is meant an amount of a pharmaceutical compound or composition having a therapeutically relevant effect in the frame of treatment and/or prevention of conditions mediated by nuclear receptors PPAR. A therapeutically relevant effect relieves to some extent one or more symptoms of conditions mediated by nuclear receptors PPAR in a patient or returns to normal either partially or completely one or more physiological or biochemical parameters associated with or causative of said conditions, e.g. increasing the sensitivity of cellular response to circulating insulin, curing, reducing, or preventing one or more clinical symptoms of PPAR related conditions, including, but not limited to, hyperglycemia, hyperinsulinemia and hypertriglyceridemia. In a preferred embodiment, a pharmaceutically effective dose of a compound or composition means an amount that increases the uptake of glucose by adipose tissue or muscle tissue. In another preferred embodiment, a pharmaceutically effective dose of a compound or composition means an amount that increases the uptake

of triglyceride by adipose tissue. The compounds of the invention are effective over a wide dosage range. For example, in the treatment of adult humans, dosages from about 0.05 to about 100 mg, preferably from about 0.1 to about 100 mg, per day may be used. A most preferable dosage is about 0.1 mg to about 70 mg per day. In choosing a regimen for patients it may frequently be necessary to begin with a dosage of about 2 to about 70 mg per day and when the condition is under control to reduce the dosage as low as from about 0.1 to about 10 mg per day. The exact dosage will depend upon the mode of administration, on the therapeutic effect that is intended to be achieved, the form in which the dosage is administered, the subject to be treated and the body weight of the subject to be treated, and the preference and experience of the physician or veterinarian in charge. Dosages and treatment schedules are readily attainable by routine experimentation to those having ordinary skill in this art. Generally, the compounds are dispensed in unit dosage form comprising from about 0.1 to about 100 mg of active ingredient together with a pharmaceutically acceptable carrier per unit dosage.

Toxicity and therapeutic efficacy of the compounds included in the compound or composition of the invention can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds which exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, special care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, leads to a reduction of side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form

employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

The route of administration of the compound or composition of the present invention may be any route, which effectively transports the active compound to the appropriate or desired site of action, such as oral, nasal, pulmonary, transdermal or parenteral e.g. rectal, depot, subcutaneous, intravenous, intraurethral, intramuscular, intranasal, ophthalmic solution or an ointment, the oral or intratumoral route being preferred.

If a solid carrier is used for oral administration, the composition may be tabletted, placed in a hard gelatin capsule in powder or pellet form or it can be in the form of a troche or lozenge. If a liquid carrier is used, the preparation may be in the form of a syrup, emulsion, soft gelatin capsule or sterile injectable liquid such as an aqueous or non-aqueous liquid suspension or solution. Tablets, dragees, or capsules having talc and/or a carbohydrate carrier or binder or the like are particularly suitable for oral application. Preferable carriers for tablets, dragees, or capsules include lactose, corn starch, and/or potato starch. A syrup or elixir can be used in cases where a sweetened vehicle can be employed.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of

ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

The invention has been described in an illustrative manner, and it is to be understood that the terminology which has been used is intended to be in the nature of words of description rather than of limitation. Obviously, many modifications and variations of the present invention are possible in light of the above teachings. It is therefore to be understood that within the scope of the appended claims, the invention may be practised otherwise than as specifically described. Accordingly, those skilled in the art will recognize, or able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described specifically herein. Such equivalents are intended to be encompassed in the scope of the following claims.

These and other embodiments are disclosed or are obvious* from and encompassed by the description and examples of the present invention. Further literature concerning any one of the methods, uses and compounds to be employed in accordance with the present invention may be retrieved from public libraries, using for example electronic devices. For example the public database "Medline" may be utilized which is available on Internet, e.g. under http://www.ncbi.nlm.nih.gov/PubMed/medline.html. Further databases and addresses, such as http://www.ncbi.nlm.nih.gov/, http://www.infobiogen.fr/, http://www.fmi.ch/biology/research_tools.html, http://www.tigr.org/, are known to the person skilled in the art and can also be obtained using, e.g., http://www.lycos.com. An overview of patent information in biotechnology and a survey of relevant sources of patent information useful for retrospective searching and for current awareness are given in Berks, TIBTECH 12 (1994), 352-364.

Figure legends

Legends

Figure 1 : Comparison of the genomic structure of the 3 isoforms of hBAF60c. Length of exons and introns of hBAF60c, in base pair number (bp) and representative scheme of the structure of hBAF60c1, hBAF60c2 and hBAF60c3. On DNA, exons (boxes) are separated by introns (lines).

Figure 2 : Study of the in vitro interaction between the N-terminal part of BAF60c and nuclear receptors.

The purified PPARγ$_E$ and RXRα$_E$ proteins were incubated with purified BAF60c2$_{1-193}$-GST or p300Nt-GST [27] and Glutathione-Q sepharose beads, either in presence of BRL 49653, a PPARγ agonist, or in presence or absence of LG1069, a RXR agonist. The beads were then washed and the samples separated on a 12% SDS-PAGE gel. Blots were developed with antibodies directed against the poly-histidine tag of PPARγ and RXRα.

Figure 3:

A. Analysis of the influence of SAF-B on PPARγ activity.

RK13 cells were transfected with an expression vector for PPARγ, increasing amounts of an expression vector for SAF-B, a reporter construct consisting of 3 PPARγ responsive elements governing the expression of the luciferase reporter gene, and a β-Galactosidase expression vector used as an internal control. Cells were then treated with a PPARγ agonist or vehicle only. After 24 hours, luciferase activity was measured and normalized to β-galactosidase expression as described in the Materials and Methods section.

B. Expression of the SAF-B protein in nuclear extracts of differentiating 3T3-L1 cells, analyzed by western blot.

Materials and Methods.

Materials.

BRL 49653 (i.e. rosiglitazone) and LG1069 are selective PPARγ and RXR agonists, respectively. The PPARγ2$_E$ protein (residues 229 to 505 of hPPARγ) and RXRα$_E$ protein (residues 225 to 462 of hRXRα). They have been produced in baculovirus and contain a labeling poly-histidine tag. Anti-$(His)_6$ antibodies were purchased at Sigma (Sigma, St. Quentin Fallavier, France).

Construction of the yeast-two hybrid library and screening.

5 µg (microg) of polyA$^+$ mRNA were extracted from human adipose tissue biopsies as described previously [20]. cDNA synthesis and cloning into Hybrizap® λ(delta) arms, phage DNA packaging, phage library amplification, "phagemid" library production, as well as library screening, and prey construct purification, were all done with Stratagene kits (Amsterdam, The Netherlands) and reactions were performed according to the manufacturer's instructions. The primary phage library obtained after packaging of the λ(delta) arms consisted in 3.10^6 independent clones. The "bait" vector was generated by cloning the DE domains of PPARγ2 (residues 179 to 505) downstream the DNA binding domain of Gal4. YRG-2 yeast (Stratagene) was sequentially transformed, first with the "bait" construct and then with the library and grown on the appropriate selective medium in presence of 1 µM BRL 49653. About 5.10^6 cotransformants were obtained. The first 100 clones which grew on the selective medium were selected for construct purification and sequencing.

Pull-down experiments.

The GST-p300Nt and GST-BAF60c2Nt fusion proteins were generated by cloning the amino-terminal parts of either the p300 protein (residues 2 to 516) or of the BAF60c2

protein (residus 1 to 193) downstream of the glutathione-S-transferase (GST) protein in the pGex-4T-1 vector (Pharmacia, Orsay, France). These fusion proteins were then expressed in *Escherichia coli* and purified on a glutathione affinity matrix (Pharmacia). The purified PPARγE or RXRαE proteins were incubated 1 hour at 22°C in pull-down buffer (phosphate-buffered saline 1x, Glycerol 10%, NP40 0,5%) with either the GST-p300Nt or the GST-BAF60c2 proteins, Glutathione-Q sepharose beads, in presence of BRL 49653 or in presence or absence of LG1069, respectively. The beads were then washed 4 times in pull-down buffer and boiled in 2x sample buffer. The samples were separated by 12% acrylamide SDS-PAGE and transferred to nitrocellulose membranes. Blots were developed with antibodies directed against the poly-histidine tag (Sigma).

Transient transfection assays

RK-13 cells (ATCC, Rockville, MD) were maintained in Dulbecco's modified Eagle's minimal essential medium (DMEM) supplemented with 10% fetal calf serum, L-glutamine, and antibiotics. Transfection with luciferase (Luc) reporter constructs was carried out as previously described [21,22]. The pGL3-$(J_{wt})_3$TKLuc [23] reporter construct contains three tandem repeats of the J site of the apolipoprotein AII promoter cloned upstream of the herpes simplex virus thymidine kinase (TK) promoter and the luciferase reporter gene. The following expression vectors were used : pSG5-hPPARγ2, a construct containing the entire cDNA of the human PPARγ2 [24]; pSG5-SAF-B, which contains the cDNA domains that interacts with S/MAR regions (1914-3018 bp) ; and pSG5, used as the control expression vector. pCMV-βGal was used as a control for transfection efficiency. Four hours after the addition of expression vectors (pSG5-PPARγ2: 100 ng/well; pSG5-SAF-B: increasing amounts), cells were grown during 20 hours in presence of BRL 49653 (10^{-8}M) or vehicle (DMSO). PPARγ activation is expressed as luciferase activity normalized to β-galactosidase activity.

Adipocyte differentiation and protein analysis

3T3-L1 cells (ATCC, Rockville, MD) were grown to confluence in medium A (Dulbecco's modified Eagle's Medium with 10% fetal calf serum, 100 units/ml penicillin, and 100µg/ml streptomycin). Confluent cell were incubated in medium A supplemented with a differentiation cocktail containing 2 µM insulin, 1 µM dexamethasone, and 0.25 mM isobuthyl methyl xanthine (Sigma) for 10 days, changing medium each other day. At different time-points, cells were lyzed and nucleus extracts were prepared as described [25]. Protein samples from these extracts were subjected to SDS-PAGE on 12% polyacrylamide gels and transferred to nitrocellulose membranes. Same quantities of proteins were loaded in each lines, as quantified by the method of Bradford [26]. Membranes were then washed and incubated overnight with a specific SAF-B antibody. Membranes were then washed for 45 min at 37°C, followed by a 1-h incubation at room temperature with anti-mouse immunoglobulin G (1:5000 dilution) conjugated to horseradish peroxidase. After washing for 45 min at 37°C, the immunoreactive bands were detected by the enhanced chemiluminesence method (ECL+, Amersham Pharmacia Biotech, Piscataway, NJ).

Example 1 : Identification of PPARγ cofactors.

In order to isolate new PPARγ regulators, the inventors have screened a human adipose tissue cDNA library in the yeast-two hybrid system, using a chimeric protein (Gal4-PPARγ2$_{179-505}$) consisting of the PPARγ2 C-terminal domain (residues 179 to 505) fused to the DNA binding domain of the yeast Gal4 activator. The original proteins or open reading frames isolated using this screening method are listed in Table I :

#	Cofactors	Identification Number
1	BRG1-associated factor 60c2	SEQ ID No 1
2	BRG1-associated factor 60c3	SEQ ID No 2
3	Scaffold attachment factor B / Hsp27 ERE-TATA-binding protein	NM_002967 U72355.1

4	Nef associated factor	NM_006058
5	Transcriptionally controlled tumor protein	NM_003295.1
6	MAP/ERK kinase 3	U78876
7	Homo sapiens genomic DNA 8p21.3 p22	SEQ ID No 3
8	Kelch motif containing protein	AB026190
9	B-Ral oncogene	X15015
10	hTu translation/elongation factor	NM_003321.1
11	protein FLJ21919	SEQ ID No 4
12	H1 histone family, member X, clone MGC:8350	SEQ ID No 5
13	Putative Ring finger protein	SEQ ID No 6
14	clone RP11-15E1 on chromosome 9	SEQ ID No 7
15	DNA from PAC 389A20	SEQ ID No 8

BAF60c

The clone #1 (SEQ ID No 1) codes for a protein identical to human BAF60c (SEQ ID NO: 11) except that the 13 first N-terminal residues of BAF60c are missing and replaced by a new domain of 26 amino acids (SEQ ID No 10). To determine whether the isolated clone could represent an alternatively spliced form of BAF60c, the inventors have first reconstituted the BAF60c genomic structure by comparing its cDNA sequence to human genome sequences available in different databases (Genbank accession numbers AC005486) and identified the intron/exon boundaries in that gene. BAF60c gene is located on chromosome 7 and comprises 13 exons and 12 introns whose sizes are indicated in Figure 1A. Comparison of the sequence of clone #1 with the one on chromosome 7 suggests that the inventors have isolated and identified a new splice variant of BAF60c, which was named BAF60c2. The N-terminal domain of clone #1 is encoded by a so far unidentified exon located between exon 1 and 2 of BAF60c (SEQ ID No 10). Hence, the inventors have named the exon encoding the N-terminal part of the BAF60c described in the literature 'exon 1A', and the newly identified exon 'exon 1B'.

The inventors have furthermore identified a second protein (clone #2) which is identical to BAF60c2 except that it lacks exon 6 to 10 as well as the first 102 nucleotides of exon

11. This protein is most probably produced from the same mRNA than BAF60c2, through an alternative splicing between exon 5 and 11 (see Figure 1). A change in the reading frame produces a protein of 200 residues (exons 12 and 13 being untranslated). This new splice variant was named BAF60c3 (SEQ ID No 2).

To characterize the interaction between these isolated BAF60c new variants and nuclear receptors, the inventors have produced a fusion protein consisting of the N-terminal domain of BAF60c2 (residues 1 to 193) and the GST protein (chimeric protein GST-BAF60c2$_{1-193}$). *In vitro* interaction between said chimeric protein and the C-terminal domains of two nuclear receptors, namely PPARγ$_E$ or RXRα$_E$, was assayed in a pull-down experiment. The C-terminal domains of these nuclear receptors encompass the ligand binding domain and, in the case of PPARγ$_2$ its ligand-dependent activation function-2.

Figure 2 illustrates that BAF60c2 interacts with the E domains of PPARγ and RXRα in presence of their respective ligands (i.e. BRL 49653 and LG1069). Additionally, Figure 2 shows that in absence of RXRα ligand, the interaction is extremely weak.

Therefore, the Inventors have shown that polypeptides including the N-terminal sequence of BAF60c2 are capable of interacting with nuclear receptors (e.g. PPARγ or RXRα). With this regard, it should be noticed that BAF60 polypeptides actually occur as part of a multiprotein chromatin complexes [28]. Several chromatin remodeling complexes, termed Swi/Snf chromatin remodeling complexes have been described (the prototypical example being the Swi2/Snf2 complex in yeast [28]). These Swi/Snf complexes all contain a central ATPase that has homology to yeast Swi2/Snf2. In mammals, two ATPases have been described to be present in the Swi/Snf complex *i.e.* BRG1 and hBrm. The Swi/Snf complexes possess an extensive repertoire of biochemical activities, ranging from nucleosome remodeling, nucleosome sliding to octamer transfer. Due to these activities, the Swi/Snf and related complexes remodel the chromatin structure and hence facilitates

the binding of transcription modulators to its DNA binding sites either resulting in a stimulation or inhibition of transcription [28-30]; thus the individual polypeptides included in said complexes are cofactors.

SAF-B

The clone #3 codes for a protein identical to SAF-B/Hsp27 ERE-TATA-binding protein (HET). This protein has been reported to bind to scaffold or matrix attachment region DNA elements (S/MAR), which are regions located near the boundaries of transcribed or silenced genes [31]. To test the role of SAF-B on PPARγ-mediated transcription, transient transfections with PPARγ and SAF-B were performed. SAF-B decreased PPARγ activity in a dose-dependent manner, in presence or absence of the synthetic PPARγ ligand BRL49653 (Figure 3A). This suggests that SAF-B acts as a ligand-independent repressor of PPARγ signaling.

PPARγ stimulates adipocyte differentiation [7]. Upon activation, PPARγ induces the transcription of genes involved in fatty acid uptake and storage in adipocytes. Expression of SAF-B was studied in 3T3-L1 cells during adipocyte differentiation. SAF-B protein levels were significantly reduced one day after addition of the differentiation cocktail, and SAF-B becomes almost undetectable after 10 days of differentiation (Figure 3B). This suggests therefore that SAF-B is a protein characteristic of the undifferentiated state, since the induction of adipocyte differentiation decreases its expression. When these expression studies are interpreted in combination with the effects of SAF-B on PPARγ activity in transient transfection assays, it becomes clear that SAF-B acts to inhibit PPARγ activity and likewise acts as an inhibitor of adipogenesis. Adipocyte differentiation requires down-regulation of SAF-B hence relieving its repressive effects ("de-repression") on the activity of PPARγ, a key coordinator of the adipocyte differentiation process.

Several models of repression of transcription by cofactors have been proposed. These models include 1) interaction with basal transcription factors and inhibition of appropriate preinitiation complex assembly; 2) down-modulation (quenching) of a transcriptional activator; 3) induction of a inactive chromatin structure [40]. It is likely that SAF-B possesses more than one of these characteristics. Firstly, SAF-B has been shown to interact with RNA polymerase II in a yeast two-hybrid system [41]. This suggests that the SAF-B could blunt the stimulation of the basal transcriptional machinery. The second evidence is that SAF-B is an abundant component of chromatin and is most likely involved in chromatin structure and remodeling. Treatment with the histone deacetylase (HDAC) inhibitor trichostatin A attenuates SAF-B-induced transcriptional repression and suggests that SAF-B could be indeed associated with HDAC complexes, which in general inhibit gene expression [39]. Finally, it was shown that SAF-B represses transcription only in the presence of S/MAR regions [41]. Hence, SAF-B could sequester nuclear receptors in the vicinity of S/MAR regions, thereby preventing their binding to their target promoters.

Some insight about, how SAF-B could affect adipogenesis could be drawn from the effects of another family of non-histone chromosomal proteins, *i.e.* proteins belonging to the high-mobility group (HMG), on adipocyte differentiation and adipogenesis [42]. Although HMG proteins do not have transcriptional activity by themselves, they change the conformation of chromatin structure and may hence influence transcription. In contrast to SAF-B, which rather act as repressors, HMG proteins most often facilitate transcription. Support for the involvement of HMGI-C in adipogenesis is provided by the observation that the mouse mutant *pygmy*, characterized by its small size and disproportionally reduced body fat content, was found to be a null allele of HMGI-C [43]. Further evidence comes from the finding that in certain lipomas gene rearrangements were found in which the HMGI-C DNA-binding domain was fused to either a LIM or an acidic transactivation domain [44,45]. In the first case, the LIM domain of the translocation partner could recruit transcriptional activators to the DNA site, whereas in the second

case, the fusion of the HMGI-C protein to an acidic transactivation domain could turn the chimeric protein into a powerful transcriptional activator. Thus, the lack or reduced expression of HMGI-C could predispose to leanness, whereas the juxtaposition of its DNA-binding motif to transcriptional regulatory domains could promote adipogenesis. Furthermore, transgenic mice carrying a truncated HMGI-C gene, which contain only the AT hook domains, develop a giant phenotype and abdominal and pelvic lipomatosis [46]. In addition to its role in regulating chromatin structure during adipogenesis, it has been shown that another member of the HMG family, *i.e.* HMG-(Y), mediates adipocyte differentiation by physically interacting with C/EBPβ, enhancing its transcriptional activity [42].

In view of these important effects of the HMG family of proteins on adipocyte homeostasis, it is then reasonable to speculate that a widely expressed, non-histone chromosomal protein such as SAF-B also affects cell differentiation in a tissue-specific manner. Transcriptional repression is part of a general strategy for the fine regulation of cell growth, metabolism, and differentiation. Specifically, the fine-tuning of the early adipocyte differentiation program requires a balance between positive (stimulatory) and negative (inhibitory) factors. We demonstrate that SAF-B might play a crucial role in inhibiting adipocyte differentiation and suggest that interfering with SAF-B activity could be a novel way to affect adipose tissue development and affect metabolic abnormalities associated with this process.

Example 2 : Human tissue specificity of the expression of the polypeptide SEQ ID NO: 12 has been determined and compared to the expression specificity of polypeptides SEQ ID NO:11.

The following table illustrates the results.

0 indicates no expression ; + indicates slight expression ; ++ indicates strong expression

Tissues	BAF60C (SEQ ID NO: 11)	BAF 60 C2 (SEQ ID NO: 12
Adrenal glands	0	0
Bone marrow	0	0
Brain	++	++
Cerebrellum	++	++
Fetal brain	++	++
Fetal liver	+	+
Heart	+	++
Kidney	+	+
Liver	0	0
Lung	0	+
Placenta	+	+
Prostate	++	+
Salivary gland	++	+
Skeletal muscle	0	++
Spleen	+	0
Testis	++	++
Thymus	0	0
Thyroid	+	++
Trachea	++	+
Uterus	++	++
Adipose tissue	0	+

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CLAIMS

1. A human polypeptide, the polypeptide comprising the N-terminal amino acid sequence set forth in SEQ ID NO:10, or a conservatively substituted variant thereof, wherein the polypeptide is a nuclear receptor cofactor .

2. The polypeptide of claim 1, consisting of all or part of the polypeptide set forth in SEQ ID No 12.

3. The polypeptide of claims 1 or 2, comprising residues 1 to 193 of the polypeptide set forth in SEQ ID No 12.

4. The polypeptide of claim 1, consisting of all or part of the polypeptide set forth in SEQ ID No 13.

5. The polypeptide of any of claims 1-4 wherein said polypeptide is a PPAR and / or a RXR cofactor.

6. The polypeptide of claim 5, wherein said polypeptide is a PPAR-gamma cofactor.

7. The polypeptide of claim 5, wherein said polypeptide is a RXR-alpha cofactor.

8. A chimeric protein comprising a polypeptide of any of claims 1-7 fused to a heterologous polypeptide.

9. The chimeric protein of claim 8, wherein said heterologous polypeptide is glutathione-S-transferase (GST).

10. A method of screening for compounds that modulate the interaction of at least one nuclear receptor with at least one nuclear receptor cofactor of any of claims 1-7, the method comprising :

(iii) incubating a reaction mixture comprising :

- at least one nuclear receptor, or fragments thereof,
- at least one potential binding modulator and,

- at least one nuclear receptor cofactor of any of claims 1-7, fragments thereof, or at least one chimeric protein of claims 8-9, and

(iv) determining whether the binding of said nuclear receptor cofactor, or of said chimeric protein, to said nuclear receptor is increased or decreased in comparison to an assay which lacks the potential binding modulator.

11. The method of claim 10, wherein the reaction mixture of step (i) comprises a fragment of the nuclear receptor, said fragment including the C-terminal domain of said receptor.

12. The method of claim 11, wherein said fragment of the nuclear receptor comprises the E domain.

13. The method of any of claims 10-12, wherein it is a screening method for compounds that enhance the interaction of said nuclear receptor with said nuclear receptor cofactor.

14. The method of any of claims 10-13, wherein it comprises a further step (iii) consisting in assessing the effect of the modulation of the binding of said nuclear receptor cofactor to said nuclear receptor as determined in step (ii) on the transactivator activity of said nuclear receptor.

15. The method of any of claims 10-14, wherein it comprises a further step (iv) consisting in determining the cell and/or tissue specificity :

- of the modulation of the binding of said nuclear receptor cofactor to said nuclear receptor as determined in step (ii), and/or

- of the effect of said modulation on the transactivator activity of said nuclear receptor as determined in step (iii).

16. The method of any of claims 10-15, wherein said nuclear receptor is the PPAR-gamma receptor.

17. The method of any of claims 10-15, wherein said nuclear receptor is the RXR-alpha receptor.

18. A method for identifying a compound that modulates at least one nuclear receptor function comprising a screening method of any of claims 8-17.

19. A method for identifying a compound that modulates the PPAR-gamma function comprising a screening method of any of claims 10-16.

20. A method for identifying a compound that modulates RXR function comprising a screening method of any of claims 10-15 or 17.

21. Compounds that modulate the interaction of at least one nuclear receptor with at least one nuclear receptor cofactor of any of claims 1-7.

22. A compound of claim 21, wherein said compound enhances the interaction of at least one nuclear receptor with at least one nuclear receptor cofactor of any of claims 1-7.

23. A compound of any of claims 21-22, wherein said compound modulates function of at least one nuclear receptor.

24. A compound of claim 23, wherein said compound enhances function of at least one nuclear receptor.

25. A compound of any of claims 21-24, wherein it is identified by a method of any of claims 10-20.

26. A compound of any of claims 21-25, wherein it is a PPAR-gamma receptor modulator.

27. A compound of any of claims 21-25, wherein it is a RXR-alpha receptor modulator.

28. A compound of any of claims 21-27, wherein said modulator is selected in the group consisting in a nuclear receptor agonists and nuclear receptor partial agonists.

29. A composition comprising at least one compound of any of claims 21-28 and a pharmaceutically acceptable carrier.

30. A method for treating and/or preventing diseases or pathologic conditions associated with cell types that express PPAR receptors comprising administering to the patient at least one compound of any of claims 21-28 or a composition of claim 29.

31. Use of at least one compound of any of claim 21-28 for the preparation of a pharmaceutical composition.

32. Use of at least one compound of any of claim 21-28 for the preparation of a pharmaceutical composition for treating and/or preventing diseases or pathologic conditions associated with cell types that express PPAR receptors.

	1A	1B	2	3	4	5	6	7	8	9	10	11	12	13
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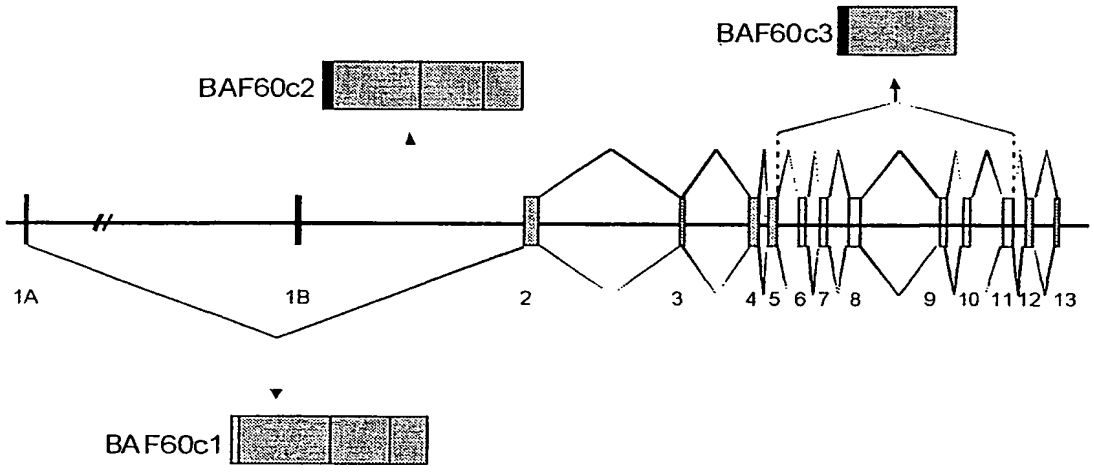

FIGURE 1

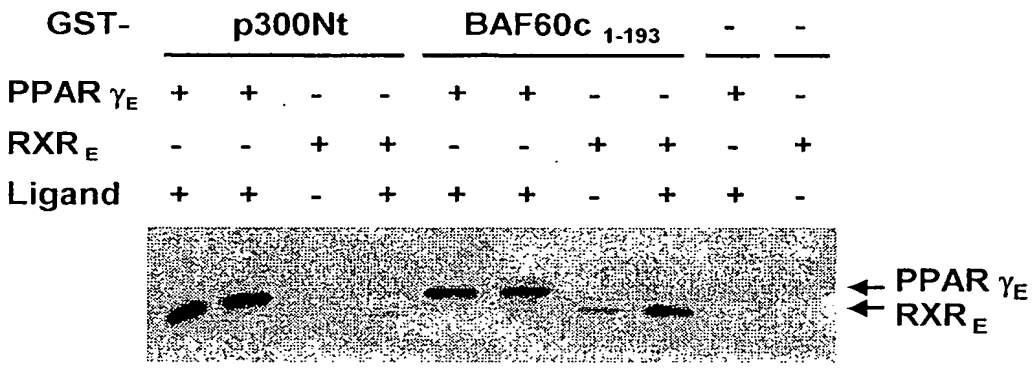

FIGURE 2

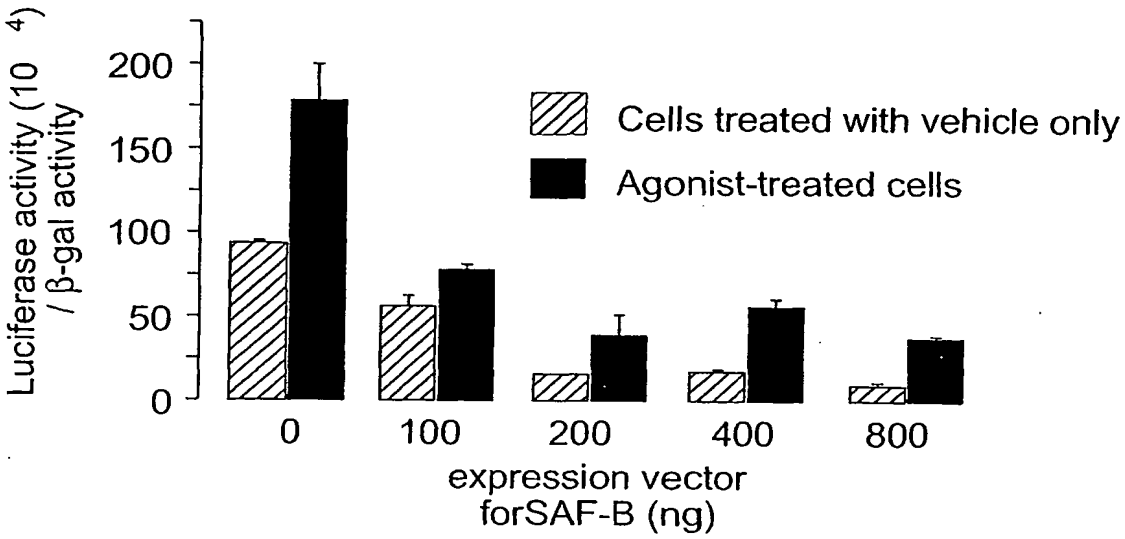

FIGURE 3A

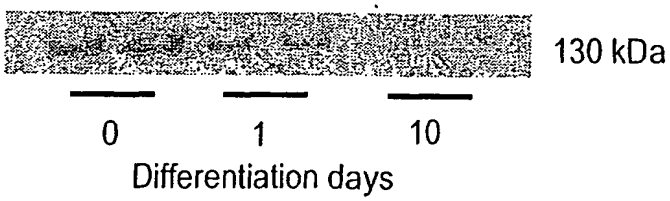

FIGURE 3B

SEQUENCE LISTING

<110> AUWERX, Johan
GELMAN, Laurent
DEBRIL, Marie-Bernard
ROCCHI, Stéphane
PICARD, Frédéric

<120> New PPARγ cofactors and medical use thereof

<130> D19558

<160> 9

<170> PatentIn Ver. 2.1

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ggcacaagcg cttctttttg gaaaacgagg agctttttg gnaacctgcn catgggganc 720
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acaggcccat agtccctgct ttgaaaacca ggggtgatga caggggccat gggtttctag 240
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SEQ ID NO:10 (N-terminal amino-acid sequence of human nuclear receptor cofactor)

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SEQ ID NO : 11 (amino-acid sequence of human nuclear receptor cofactor BAF 60c)

Met thr pro gly leu gln his pro pro thr val val gln arg pro gly met pro ser gly
ala arg met pro his gln gly ala pro met gly pro pro gly ser pro tyr met gly ser
pro ala val arg pro gly leu ala pro ala gly met glu pro ala arg lys arg ala ala
pro pro pro gly gln ser gln ala gln ser gln gly gln pro val pro thr ala pro ala
arg ser arg ser ala lys arg arg lys met ala asp lys ile leu pro gln arg ile arg
glu leu val pro glu ser gln ala tyr met asp leu leu ala phe glu arg lys leu asp
gln thr ile met arg lys arg val asp ile gln glu ala leu lys arg pro met lys gln
lys arg lys leu arg leu tyr ile ser asn thr phe asn pro ala lys pro asp ala glu
asp ser asp gly ser ile ala ser trp glu leu arg val glu gly lys leu leu asp asp
pro ser lys gln lys arg lys phe ser ser phe phe lys ser leu val ile glu leu asp
lys asp leu tyr gly pro asp asn his leu val glu trp his arg thr pro thr thr gln
glu thr asp gly phe gln val lys arg pro gly asp leu ser val arg cys thr leu leu
leu met leu asp tyr gln pro pro gln phe lys leu asp pro arg leu ala arg leu leu
gly leu his thr gln ser arg ser ala ile val gln ala leu trp gln tyr val lys thr
asn arg leu gln asp ser his asp lys glu tyr ile asn gly asp lys tyr phe gln gln
ile phe asp cys pro arg leu lys phe ser glu ile pro gln arg leu thr ala leu leu
leu pro pro asp pro ile val ile asn his val ile ser val asp pro ser asp gln lys
lys thr ala cys tyr asp ile asp val glu val glu glu pro leu lys gly gln met ser
ser phe leu leu ser thr ala asn gln gln glu ile ser ala leu asp ser lys ile his
glu thr ile glu ser ile asn gln leu lys ile gln arg asp phe met leu ser phe ser
arg asp pro lys gly tyr val gln asp leu leu arg ser gln ser arg asp leu lys val
met thr asp val ala gly asn pro glu glu glu arg arg ala glu phe tyr his gln pro
trp ser gln glu ala val ser arg tyr phe tyr cys lys ile gln gln arg arg gln glu
leu glu gln ser leu val val arg asn thr

SEQ ID NO : 12 (amino-acid sequence of human nuclear receptor cofactor BAF 60c2)

Met ala ala asp glu val ala gly gly ala arg lys ala thr lys ser lys leu phe glu
phe leu val his gly val arg pro gly met pro ser gly ala arg met pro his gln gly

41 51
ala pro met gly pro pro gly ser pro tyr met gly ser pro ala val arg pro gly leu
61 71

ala pro ala gly met glu pro ala arg lys arg ala ala pro pro pro gly gln ser gln
81 91
ala gln ser gln gly gln pro val pro thr ala pro ala arg ser arg ser ala lys arg
101 111
arg lys met ala asp lys ile leu pro gln arg ile arg glu leu val pro glu ser gln
121 131
ala tyr met asp leu leu ala phe glu arg lys leu asp gln thr ile met arg lys arg
141 151
val asp ile gln glu ala leu lys arg pro met lys gln lys arg lys leu arg leu tyr
161 171
ile ser asn thr phe asn pro ala lys pro asp ala glu asp ser asp gly ser ile ala
181 191
ser trp glu leu arg val glu gly lys leu leu asp asp pro ser lys gln lys arg lys
201 211
phe ser ser phe phe lys ser leu val ile glu leu asp lys asp leu tyr gly pro asp
221 231
asn his leu val glu trp his arg thr pro thr thr gln glu thr asp gly phe gln val
241 251
lys arg pro gly asp leu ser val arg cys thr leu leu leu met leu asp tyr gln pro
261 271
pro gln phe lys leu asp pro arg leu ala arg leu leu gly leu his thr gln ser arg
281 291
ser ala ile val gln ala leu trp gln tyr val lys thr asn arg leu gln asp ser his
301 311
asp lys glu tyr ile asn gly asp lys tyr phe gln gln ile phe asp cys pro arg leu
321 331
lys phe ser glu ile pro gln arg leu thr ala leu leu leu pro pro asp pro ile val
341 351
ile asn his val ile ser val asp pro ser asp gln lys lys thr ala cys tyr asp ile
361 371
asp val glu val glu glu pro leu lys gly gln met ser ser phe leu leu ser thr ala
381 391
asn gln gln glu ile ser ala leu asp ser lys ile his glu thr ile glu ser ile asn
401 411
gln leu lys ile gln arg asp phe met leu ser phe ser arg asp pro lys gly tyr val
421 431
gln asp leu leu arg ser gln ser arg asp leu lys val met thr asp val ala gly asn
441 451
pro glu glu glu arg arg ala glu phe tyr his gln pro trp ser gln glu ala val ser
461 471
arg tyr phe tyr cys lys ile gln gln arg arg gln glu leu glu gln ser leu val val
481
arg asn thr

SEQ ID NO:13 (amino-acid sequence of human nuclear receptor cofactor BAF 60c3)

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phe leu val his gly val arg pro gly met pro ser gly ala arg met pro his gln gly
ala pro met gly pro pro gly ser pro tyr met gly ser pro ala val arg pro gly leu
ala pro ala gly met glu pro ala arg lys arg ala ala pro pro pro gly gln ser gln
ala gln ser gln gly gln pro val pro thr ala pro ala arg ser arg ser ala lys arg
arg lys met ala asp lys ile leu pro gln arg ile arg glu leu val pro glu ser gln
ala tyr met asp leu leu ala phe glu arg lys leu asp gln thr ile met arg lys arg
val asp ile gln glu ala leu lys arg pro met lys gln lys arg lys leu arg leu tyr
ile ser asn thr phe asn pro ala lys pro asp ala glu asp ser asp gly ser ile ala
ser trp glu leu arg val glu gly lys leu leu asp asp pro arg ala gly thr ser arg
gln met pro ala thr leu lys arg ser ala gly leu ser ser thr thr ser pro gly pro
arg arg pro ser val ala thr ser thr ala arg ser ser ser ala gly arg ser trp ser
ser arg trp leu cys ala thr pro

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(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
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(72) Inventors; and

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:
— *of inventorship (Rule 4.17(iv)) for US only*

Published:
— *with international search report*

(88) Date of publication of the international search report: 10 June 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: NEW NUCLEAR RECEPTOR COFACTORS AND RELATED MODULATORS

(57) Abstract: The invention relates to nuclear receptor cofactors, to methods for identifying modulators of nuclear receptor activity and to methods for treating and/or preventing or pathologic conditions associated with cell types that express said nuclear receptors. In particular, the invention concerns cofactors of PPAR-gamma (PPARγ), screeing methods that can be used for the identification of compounds useful for modulating the PpAR-gamma biological activity and methods of treatment and/or prevention of various PPAR-gamma related diseases and conditions, including metabolic or cell proliferative disorders.

WO 2002/094877 A3

INTERNATIONAL SEARCH REPORT

Inte[illegible]nal Application No
PCT/IB 02/02939

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C07K14/47 C12N15/62 G01N33/50

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, EMBL, MEDLINE, WPI Data, BIOSIS, EMBASE, SEQUENCE SEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ZHU Y ET AL: "Isolation and characterization of peroxisome proliferator-activated receptor (PPAR) interacting protein (PRIP) as a coactivator for PPAR." THE JOURNAL OF BIOLOGICAL CHEMISTRY. UNITED STATES 5 MAY 2000, vol. 275, no. 18, 5 May 2000 (2000-05-05), pages 13510-13516, XP002245759 ISSN: 0021-9258 cited in the application the whole document --- -/--	1-30

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 July 2003	11/07/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Piret, B

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ZHU Y ET AL: "ISOLATION AND CHARACTERIZATION OF PBP, A PROTEIN THAT INTERACTS WITH PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 272, no. 41, 10 October 1997 (1997-10-10), pages 25500-25506, XP002915089 ISSN: 0021-9258 the whole document ---	1-30
A	RING H Z ET AL: "Five SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin (SMARC) genes are dispersed in the human genome" GENOMICS, ACADEMIC PRESS, SAN DIEGO, US, vol. 51, no. 1, 1 July 1998 (1998-07-01), pages 140-143, XP002223620 ISSN: 0888-7543 the whole document ---	1-9
A	HANKE J ET AL: "Alternative splicing of human genes: more the rule than the exception?" TRENDS IN GENETICS, ELSEVIER SCIENCE PUBLISHERS B.V. AMSTERDAM, NL, vol. 15, no. 10, 1 October 1999 (1999-10-01), pages 389-390, XP004179550 ISSN: 0168-9525 the whole document ---	1-9
A	DATABASE GENBANK 'Online! 22 May 2001 (2001-05-22) NIH-MGC: "NIH_MGC_17 Homo sapiens cDNA clone IMAGE:4900666 5', mRNA sequence" retrieved from NCBI Database accession no. BG825152 XP002245760 the whole document ---	1-3
A	DATABASE GENBANK 'Online! 8 February 2001 (2001-02-08) "Mus musculus adult male cerebellum cDNA, clone:1500001J14" retrieved from NCBI Database accession no. AK005094 XP002245761 the whole document --- -/--	1-3

INTERNATIONAL SEARCH REPORT

Int onal Application No
PCT/IB 02/02939

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	COLLINGWOOD T N ET AL: "NUCLEAR RECEPTORS: COACTIVATORS, COREPRESSORS AND CHROMATIN REMODELING IN THE CONTROL OF TRANSCRIPTION" JOURNAL OF MOLECULAR ENDOCRINOLOGY, XX, XX, vol. 23, 1999, pages 255-275, XP001050651 ISSN: 0952-5041	

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB 02/02939

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claim 30 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 21-32 (all completely)
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 21-32 (all completely)

Present claims 21-32 relate to a compound defined by reference to a desirable characteristic or property, namely the ability to modulate the interaction of at least one nuclear receptor with at least one nuclear receptor cofactor of claims 1-7.

The claims cover all compounds having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a limited number of such compounds (BRL49653 and LG1069). In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compound by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the compounds BRL49653 and LG1069 and uses thereof.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.